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Introduction to Biotechnical and Biomedical Applications of Poly(Ethylene Glycol)

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1.1. INTRODUCTION

At first glance, the polymer known as poly(ethylene glycol) or PEG appears to be a simple molecule. It is a linear or branched, neutral polyether, available in a variety of MWs, and soluble in water and most organic solvents. Despite its apparent simplicity,

$$HO—(CH_2CH_2O)_n—CH_2CH_2OH$$

poly(ethylene glycol)

this molecule is the focus of much interest in the biotechnical and biomedical communities. Primarily this is because PEG is unusually effective at excluding other polymers from its presence when in an aqueous environment. This property translates into protein rejection, formation of two-phase systems with other polymers, non-immunogenicity, and nonantigenicity. In addition, the polymer is nontoxic and does not harm active proteins or cells although it interacts with cell membranes. It is subject to ready chemical modification and attachment to other molecules and surfaces, and when attached to other molecules it has little effect on their chemistry but controls their solubility and increases their size. These properties, which are described in more detail below, have led to a variety of important biotechnical and biomedical applications, a summary of which is also presented below.

Five key early works set the stage for the applications described in this volume; without regard to any order these are: (1) the observation that PEG can be used to

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Poly(Ethylene Glycol) Chemistry: Biotechnical and Biomedical Applications, edited by J. Milton Harris. Plenum Press, New York, 1992.

drive proteins and nucleic acids from solution for purification and crystal growth[1–3]; (2) Albertsson's discovery that PEG and dextran, when mixed with buffer, form aqueous polymer two-phase systems, which are hospitable to biological materials and are extremely useful for purification of these biological materials[4]; (3) the finding that PEG interacts with cell membranes to give cell fusion, a key process in biotechnology[5–7]; (4) Davis and Abuchowski's observation that covalent attachment of PEG to proteins gives active conjugates that are nonimmunogenic and nonantigenic and have greatly increased serum lifetimes[8]; and (5) Nagaoka's finding that covalent attachment of PEG to surfaces greatly retards protein adsorption to these surfaces.[9]

These early works have led to several active areas of investigation, which have produced hundreds of research publications. The present volume is the first to bring these diverse applications together in one source. A major goal of this book is to show the interrelationships among these different areas and thus stimulate new areas of investigation.

1.2. PROPERTIES OF PEG

At molecular weights less than 1000, PEGs are viscous, colorless liquids; higher molecular weight PEGs are waxy, white solids.[10,11] The melting point of the solid is proportional to molecular weight, approaching a plateau at about 67 °C. The molecular weights commonly used in biomedical and biotechnical applications range from a few hundred to approximately 20,000. Since PEG is usually prepared by an anionic initiation process with few chain-transfer and termination steps, molecular weight distributions are generally observed to be narrow.[9] It should be noted, however, that the commonly used monomethyl ethers of PEG exhibit a rather broad molecular weight distribution because of the presence of high molecular weight PEG (i.e., the polymer with two hydroxyl terminal groups).[12] This PEG is produced from trace hydroxide acting as an initiator, and since this difunctional polymer grows at both ends, it has a higher molecular weight than the monomethyl ether, which grows at only one end.

PEGs are also sometimes referred to as poly(ethylene oxide) (PEO), poly(oxyethylene) (POE), and polyoxirane. In general usage, poly(ethylene glycol) refers to polyols of molecular weights below about 20,000, poly(ethylene oxide) refers to higher molecular weight polymers, and poly(oxyethylene) and poly(oxirane) are not specific in this regard. The *Chemical Abstracts* registry number for PEG is 25322-68-3.

PEGs possess a variety of properties pertinent to biomedical and biotechnical applications. A summary of these properties is given here, with applications described in Section 1.3. The following is a brief listing of some properties of interest.

1. Soluble in water, toluene, methylene chloride, many organic solvents.
2. Insoluble in ethyl ether, hexane, ethylene glycol.

3. Insoluble in water at elevated temperature.
4. Solubility and partitioning controlled by making derivatives.
5. Forms complexes with metal cations.
6. Highly mobile; large exclusion volume in water.
7. Can be used to precipitate proteins and nucleic acids.
8. Forms two-phase systems with aqueous solutions of other polymers.
9. Nontoxic; FDA approved for internal consumption.
10. Hospitable to biological materials.
11. Causes cell fusion (in high concentration).
12. Weakly immunogenic.

If covalently linked PEG will:

13. Solubilize other molecules.
14. Render proteins nonimmunogenic and toleragenic.
15. Reduce rate of clearance through kidney.
16. Render surfaces protein-rejecting.
17. Alter electroosmotic flow.
18. Move molecules across cell membranes.
19. Alter pharmacokinetics.

1.2.1. Solubility and Partitioning

PEG exhibits a bizarre solubility pattern, as it is soluble both in water and (to varying degrees) in many organic solvents including toluene, methylene chloride, ethanol, and acetone (hence PEG is frequently described as amphiphilic).[10] Interestingly, the closely related poly(methylene oxide), poly(propylene oxide), and isomeric polyacetaldehyde are not soluble in water. PEG is insoluble in hexane and similar aliphatic hydrocarbons and in ethyl ether and ethylene glycol, molecules which closely resemble PEG. This solubility pattern is of much use in synthesis of PEG derivatives since reactions can be conducted in an organic solvent, such as toluene, and the product isolated by addition to a nonsolvent such as hexane or ethyl ether. The Hildebrand solubility parameter is 10.3 $(\text{cal cm}^{-3})^{0.5}$ for PEG.[11]

Since PEG is soluble in both organic and aqueous media, it is apparent that the polymer will be present to some extent in both phases of an organic-water, two-phase system. Although this partitioning has not been studied in great detail, it is known that PEG will partition in favor of water in a water–benzene system and in favor of methylene chloride in a water–methylene chloride system. A common isolation procedure in synthesis of PEG derivatives is to extract the derivative into water and then into methylene chloride.

In biological systems, it appears that PEG partitions between aqueous medium and cell membranes. Evidence for this lies in the observation that PEG induces cell fusion, a property used to great benefit in production of hybridomas and monoclonal antibodies.[5–7] This topic is discussed in Section 1.3.5.

PEG solubility and partitioning patterns can be altered by attachment of hydrophobic tails, as in the common PEG-based surfactants, and by including hydrophobic comonomers in the polymer backbone, as in the common ethylene oxide–propylene oxide copolymers (inclusion of greater than 50% propylene oxide will make the polymer water-insoluble).[10] This alteration in solubility can be used to control partitioning of PEG derivatives in two-phase systems, such as benzene versus water, where attachment of hydrocarbon tails shifts partitioning in favor of the organic layer.[13]

PEGs also have the unusual property of possessing a lower consolute temperature (LCT), or cloud point, of approximately 100 °C in water; that is, raising the temperature above 100 °C will result in insolubility and formation of two phases.[10,11] The LCT for PEG varies somewhat depending on molecular weight, concentration, and pH. Increasing salt concentration can greatly lower the LCT. Also, inclusion of propylene oxide comonomer lowers the LCT proportionately, until the polymer with 60% ethylene oxide and 40% propylene oxide becomes insoluble at 37 °C. Attachment of hydrophobic end groups, as in the large number of PEG-based surfactants, has a similar effect. This inverse solubility–temperature relationship in water has several practical applications.

Finally, PEG solubility properties are revealed in the many uses of PEG as a copolymer in a variety of block copolymers (see Chapter 14 by Merrill on PEG-polyurethanes) and in polymer blends.[11] In the block copolymers the PEG can form an immiscible "soft segment" phase which becomes the phase in contact with an aqueous medium. Similarly, the blends can be miscible or immiscible blends. Polymer miscibility is a complex phenomenon in which changes in thermodynamic parameters are important. A discussion of this subject is given by Bailey and Koleske.[11]

1.2.2. Metal Binding

PEGs form complexes with metal cations.[10,11] This property is demonstrated by application of PEGs as "phase transfer agents," a process of much importance in organic chemistry.[13–15] In this application the PEG transfers a salt from solid phase or aqueous phase to organic phase by complexing or coordinating with the metal cation and assisting its partition into the organic phase. The corresponding anion has to follow along to maintain charge neutrality, and in the process the anion becomes much more reactive because it is poorly solvated or "naked"; hence, this process is frequently referred to as phase transfer catalysis. Examples include movement of $KMnO_4$ from solid phase into benzene (using an alkyl-PEG) and transfer of metal picrates from water into methylene chloride (using PEG itself).[13–15]

It is noteworthy that phase transfer catalysis was first described for "crown ethers," which are cyclic ethers resembling rings made of PEG of approximately six ethylene oxide units.[16] The molecular structures of these ethers resemble crowns in which oxygens lie at the points of the crown. Complexation occurs when an electron-

deficient metal cation coordinates with the electron-rich oxygens. After complexation, the metal is rendered hydrophobic and organic soluble, because it is hidden inside the hydrocarbon portion of the crown ether. Crown ethers show selectivity in metal binding related to the size of the cavity in the center of the crown. Interestingly, PEGs also show selectivity in metal binding, apparently because the PEGs adopt helical conformations with cavities of preferred sizes.[11,14]

1.2.3. Aqueous Solutions

The behavior of PEG in aqueous environments is the key to its importance in biomedical and biotechnical applications. In simple terms, PEG in aqueous solution acts as a highly mobile molecule with a large exclusion volume. Relaxation-time studies show rapid motion of the polymer chain[17] and gel chromatography shows that PEGs are much larger in solution than many other molecules (e.g., proteins) of comparable molecular weight.[18,19] Interesting consequences of this property are that PEG excludes other polymers and, if the concentration of PEG is high enough, will form two-phase systems with other polymers. Applications that result include protein and nucleic acid precipitation and two-phase partitioning and, when the PEG is covalently bound, protein-rejecting molecules and surfaces. Also, as noted in the discussion of solubility (Section 1.2.1), incorporation of hydrophobic end groups, inclusion of hydrophobic propylene oxide as comonomer, and addition of salts lead to lowering of the lower consolute temperature; this phenomenon has been utilized to produce two-phase systems for partitioning purifications.

Molecular modeling and theoretical investigations of PEG solution properties and interactions with other polymers are discussed in Chapters 2, 3, and 15. Theoretical investigation of formation of two-phase systems has also been the subject of several recent studies.[20–23]

1.2.4. Covalently Bound PEGs

The terminal hydroxyl groups of the PEG molecule provide a ready site for covalent attachment to other molecules and surfaces.[24] Molecules to which PEG is attached usually remain active, demonstrating that bound PEG does not denature proteins or hinder the approach of other small molecules. Bound PEG does, however, retain its ability to repel other large molecules, and thus PEG-modified surfaces and PEG-modified proteins are protein-rejecting. Consequently, there are many examples of PEG-enzymes with small substrates that remain active.[24,25] Although PEG-protein interactions with large molecules have not yet been investigated in great detail, it is known that some interactions can occur. For example, we have shown that the PEG-modified antibody against alkaline phosphatase will continue to bind with alkaline phosphatase[26] and that the PEG-modified antibody against red blood cells will continue to bind with red blood cells.[27] Similarly, a variety of PEG-bound affinity ligands (both protein and nonprotein) used in two-phase partitioning are known to

continue binding with their substrates (see Chapters 4, 5, and 6). On the other hand, the well-known reduction of immunogenicity and antigenicity for PEG-modified proteins and the nonfouling nature of PEG-modified surfaces must derive in large part from the inability of nonbound proteins to approach these materials. Obviously more work is needed to give a better understanding of interaction of PEG-modified moieties with other molecules.

Workers investigating protein-rejecting surfaces have conducted experiments to elucidate better the nonfouling nature of PEG-surfaces (see Chapters 14–16, and 18). We give here a qualitative view drawn from this volume and from our recent work.[28] As noted above, PEG in aqueous solution is a highly mobile molecule with a large exclusion volume and, of course, the molecule is neutral and possesses no acidic sites (excluding the hydroxyl group which acts as a weak hydrogen-bond acid) and only weakly basic ether linkages. The molecule is also heavily hydrated. One immediate conclusion is that there are few sites to which proteins can bind. Moreover, the rapid motion of the molecule gives an approaching protein little time in which to form a positive interaction.[17]

Additionally, consider what happens if a protein senses and binds with the surface beneath the PEG. There can be two consequences. First, if the PEG molecules are compressed against the surface, without losing water molecules, there will be an unfavorable negative entropy change resulting from the reduced motion of the highly mobile, hydrated chain. This entropic disadvantage can be overcome if water molecules are stripped from the PEG chains, but this in turn is enthalpically unfavorable. Since protein adsorption to PEG-modified surfaces is minimal, it is apparent that neither of these consequences obtains.

Thus PEG molecules bound to surfaces can be viewed as being mobile, heavily hydrated, elastic spheres. A protein interacting with these spheres can temporarily deform the sphere but cannot attach to or significantly compress the sphere. A dependence of this phenomenon on molecular weight has been observed and is discussed in Chapter 2.

Molecules bound to PEG have altered solubility properties. Thus, PEG attachment may be used to improve water solubility. Similarly, PEG binding can improve solubility of molecules in organic solvents, and this property has been utilized to make PEG-enzymes that are active in dry organic solvents (Section 1.3.3). In view of these applications, and the solubility of PEGs and PEG conjugates in water and organic solvents, it would be expected that PEG could be used to move molecules across cell membranes. Preliminary evidence that this can in fact be accomplished is presented in Section 1.3.5.

Covalent linkage of PEGs also increases the size of the molecule to which the PEG is bound, and this property has been utilized to reduce the rate of clearance of molecules through the kidney (Section 1.3.2). Finally, covalent linkage of PEGs alters the electrical nature of surfaces since charges on the surface become buried beneath a viscous, hydrated, neutral layer. This property has been utilized in capillary electrophoresis to control electroosmotic flow (Section 1.3.3).

1.2.5. Toxicity

Of much interest in the biomedical area is the fact that PEG is nontoxic and has been approved by the FDA for internal consumption.[29–31] PEG is used in large quantities for drug compounding and for a wide variety of cosmetic and personal care products, and PEG-proteins have been cleared for clinical trials in humans (Section 1.3.2). It is of interest that small PEGs of molecular weight less than 400 may exhibit some toxicity.[29] Free PEG administered intravenously to humans is readily excreted through the kidney.[32]

PEG is poorly immunogenic, which, of course, is crucial to the development of PEG-proteins as drugs (Section 1.3.2).[33] Richter and Akerblom have studied antibody formation in humans exposed to PEG and PEG-proteins, and find that PEG is poorly immunogenic, while PEG-proteins can elicit a mild anti-PEG response.[34,35] Interestingly, this response dwindles with increased exposure time, and is sufficiently weak to be of no clinical significance.

Finally, it is noteworthy that aqueous solutions of PEG are hospitable to living cells. This fact has long been appreciated by experimentalists dealing with aqueous polymer two-phase systems,[4] and PEG is utilized in tissue culture media and for organ preservation.

1.3. BIOTECHNICAL AND BIOMEDICAL APPLICATIONS OF PEG

The purpose of this section is to provide a brief review of applications and especially to show how the different chapters of this book are related. Obviously this short review cannot be exhaustive, but an attempt has been made to provide leading references to primary sources, and of course many references can be found in the individual chapters. A recent review by Topchieva contains many additional references.[36]

1.3.1. Biological Separations

This volume includes three chapters on the "phase partitioning" technique first developed by Albertsson.[4] The formation of aqueous polymer two-phase systems has been known since the 19th century, but it remained for Albertsson to utilize the phase systems for biological purifications. The procedure involves purification of biological materials (cells, cell fragments, viruses, proteins, nucleic acids) by partitioning between the two phases formed by solution of a pair of polymers (typically PEG and dextran) in aqueous buffer. It is indeed interesting that immiscible layers can be formed, although each layer is composed of more than 90% water. As noted earlier, this incompatibility of PEG with other polymers is one of the key properties of PEG.

The physical mechanism for this incompatibility is not clear (it has been

examined in terms of Flory–Huggins theory),[20–23] but the incompatibility of PEG with neutral polymers, such as dextran, is clearly related to its incompatibility with proteins and nucleic acids that makes possible nonimmunogenic enzymes and nonfouling surfaces. It should be noted that PEG is not unusual in formation of two-phase systems; many pairs of polymers exhibit this phenomenon, and it is even possible to form multilayered systems by simultaneous solution of several polymers. Salt solutions also form two-phase systems with PEG and water (the salt essentially lowers the lower consolute temperature), and PEG will form a two-phase system with pure water above its lower consolute temperature.

A detailed discussion of many aspects of phase partitioning follows in Chapters 3–6, but it is pertinent to summarize a few key points regarding the technique. Partitioning of materials between the two phases, or between the phases and the phase interface in the case of particles, can be controlled by varying phase components including salt concentration, salt identity, pH, polymer identity, polymer MW, and phase-volume ratio. It is especially noteworthy that increasing PEG MW acts to drive proteins to the dextran-rich phase, while increasing dextran MW acts to drive proteins to the PEG-rich phase. Also, certain salts give "neutral" phase systems in which purifications are not sensitive to the charge of the desired protein or cell but depend rather on subtle factors such as variation in membrane lipids. Other salts, however, are not partitioned equally between the phases and thus give "charged" phase systems in which purifications are dependent on charge of the target moiety.

Affinity partitioning can be performed by covalent attachment of affinity ligands to PEG. Ligands that have been utilized include dyes, antibodies, enzyme inhibitors, metals, and hydrophobic alkyl chains. The target substance can be separated from the affinity ligand by the usual pH and salt variations or, if an ethylene oxide–propylene oxide copolymer is used, by heating above the lower consolute point of the polymer.[37] Affinity partitioning separations offer powerful competition for other affinity techniques such as cell sorting and chromatography. Frequently, the technique is applied by use of multiple separations in the form of countercurrent distribution (repeated single-tube separations) or countercurrent chromatography (in which the two immiscible liquids flow past each other).

Finally, it is important to note that phase partitioning offers many advantages for large-scale, industrial purifications. The technique can be scaled up directly, and many techniques are available for handling liquid–liquid, two-phase systems. The inexpensive polymers can be recovered and recycled after use. An important advantage for work with cell homogenates (e.g., in recombinant DNA technology) is that cell debris collects at the phase interface while nucleic acids can be collected in the bottom phase. Thus partitioning is especially attractive as a first step in large-scale recovery of proteins.

There are several variations on this two-phase partitioning theme. For example, PEG bis-copper-chelates can be used for metal-affinity precipitation of proteins.[38] The PEG-derived detergent Triton X-114 can be dissolved in buffer, mixed with a crude protein mixture, and heated above the cloud point to form a two-phase system and partition the proteins.[39] Also, a chromatographic form of partitioning can be derived

by immobilizing the dextran–water phase on a chromatographic support and eluting with the PEG-water phase.[40]

1.3.2. PEG-Proteins and PEG-Peptides for Medical Applications

In 1977, Davis, Abuchowski, and co-workers demonstrated that covalent attachment of PEG to a protein gives minimal loss of activity and renders the protein nonimmunogenic and nonantigenic, thus imparting greatly increased serum lifetime.[8,33] This observation is highly significant because it opens the way to intravenous administration of proteins for treatment of inborn errors of metabolism and other disorders. Additional benefits of protein "pegylation" are reduced rate of kidney clearance and enhanced association with cells (see Section 1.3.5).[41] Examples of medically useful PEG-modified proteins include PEG-asparaginase for treatment of acute leukemias,[42] PEG-adenosine deaminase for treatment of severe combined immunodeficiency disease,[43] and PEG-superoxide dismutase and PEG-catalase for limiting tissue injury resulting from reactive oxygen species associated with ischemia and related pathological events.[41] In the present volume, Chapter 21 describes synthetic methods for attaching PEG to proteins, Chapter 9 describes several useful applications of PEG-proteins including PEG-superoxide dismutase, Chapter 11 describes use of PEG-bilirubin oxidase for treatment of jaundice, Chapter 12 describes PEG-hemoglobin as a whole blood substitute, and Chapter 13 describes use of PEG-collagen for soft tissue replacement.

Lightly pegylated protein allergens can be used in hyposensitization of allergic patients.[44] Work in this area is described in Chapter 10.

There is also much pharmaceutical interest in PEG modification of small drug molecules (penicillin, procaine, aspirin, etc.) because of enhanced water solubility, altered pharmacokinetics, and greatly increased size and reduced rate of renal clearance.[45] For example, there is interest in using PEG-peptides, such as PEG-interleukin 2, for stimulation of the immune system.[46]

1.3.3. PEG-Proteins for Chemical/Biotechnical Applications

In addition to the medical and separations applications of PEG-proteins described above, these conjugates have other applications in biotechnology. In particular it is noteworthy that PEG attachment induces solubility of PEG-enzymes in dry organic solvents, such as chloroform, where they exhibit interesting properties.[47]

Another application of PEGs is in synthesizing artificial enzymes by connecting binding sites and catalytic sites via PEG linkers. The PEG linker is a key aspect of this application because of its length, hydrophilicity, and flexibility. In Chapter 8, Yomo, Urabe, and Okada describe application of this concept in preparing semisynthetic oxidases. In a related work Yoshinaga, Ishida, Sagawa, and Ohkubo (Chapter 7) have coupled catalytically active metal–porphyrin complexes to PEG-modified BSA, which acts as a binding site, to obtain hybrid catalysts that are active in organic media.

1.3.4. PEG on Surfaces

1.3.4.1. Nonfouling Surfaces

There is a great deal of current interest in using surface-bound PEG for preventing protein adsorption. This topic is discussed in Chapters 14–16 and a qualitative discussion of the mechanism for protein rejection was given above in Section 1.2.4. Attaching a monolayer of PEG to a surface can reduce the likelihood of many medically undesirable processes. Drug delivery from PEG-based gels, which also exhibit excellent protein-rejecting properties, shows much promise as well; this topic is discussed in Chapter 17.

In a related application, recent work has shown that incorporation of PEG-lipids into the surface of liposomes gives increased serum lifetime and altered pharmacokinetics.[48,49] Thus it appears that PEG on the surface of the liposome acts, as in the case of PEG-proteins, to shield the small particles from the immune system.

1.3.4.2. As a Tether between Molecules and Surfaces

PEG exhibits interesting properties when used as a tether or linker to couple an active molecule to a surface. In this application the PEG acts to inhibit nonspecific protein adsorption on the surface. Additionally, the tethered molecules have been shown to be highly active, behaving essentially as free molecules in solution. This observation can be rationalized by assuming that the unusually long, well-hydrated PEG linker moves the active molecule well out into solution, some distance from the surface.

Applications of this concept to extracorporeal devices, peptide synthesis (see Bonora *et al.* for related oligonucleotide synthesis),[50] diagnostics, and blood contacting materials are described in Chapters 18–20.

1.3.4.3. For Control of Electroosmosis

Electrosmosis is the fluid flow adjacent to a charged surface that results (for example, in capillary electrophoresis) when an electrical potential is applied, causing soluble counterions to migrate toward the oppositely charged electrode. Workers in capillary electrophoresis have long been interested in eliminating or controlling electroosmosis. One approach to solving this problem has been to adsorb polysaccharides onto the charged surface to produce a viscous layer which impedes counterion movement. A problem with this approach is that the polysaccharides tend to desorb, especially in the presence of proteins. We showed in 1986 that covalently bound PEG-5000 essentially eliminates electroosmotic flow, while lower molecular weight PEGs reduce such flow.[51,52] Since our early work there has been a resurgence of interest in capillary electrophoresis as an analytically technique and other workers have examined PEG coatings.[53] An additional benefit of the PEG coatings is that protein adsorption to the capillary surface is also greatly reduced.

1.3.5. Cell Membrane Interactions

The amphiphilic nature of PEG indicates that PEG might interact with cell membranes but the details of these interactions remain relatively poorly understood. Evidence that this interaction occurs is the well-known PEG-induced fusion of cells and liposomes.[5–7,54] Although the mechanism of this process remains debatable, it is clear that membranes absorb large quantities of PEG and that PEG–membrane interaction is probably involved in membrane fusion.[54,55]

This membrane-associative behavior of PEG encouraged Beckman *et al.* to examine endothelial cell uptake of PEG-superoxide dismutase and catalase.[41] These workers found that the PEG-enzymes were in fact taken up by the cells and remained active within the cells. Three mechanisms were proposed for this uptake: (1) direct penetration of membranes, (2) binding of PEG-enzymes to membrane surfaces, and (3) uptake by endocytosis. The first mechanism was considered to be unlikely because PEG is insoluble in alkanes, and thus the PEG-enzyme would not be expected to be soluble in the aliphatic core of phospholipid membranes. However, PEG is known to associate with the phospholipid head-group region of membranes.[54] This fact plus the observed kinetics of uptake and the enhancement of uptake following mechanical injury of the endothelial cell membrane are consistent with membrane binding followed by endocytosis.

In a related work, Veronese and co-workers noted that PEG-superoxide dismutase, unlike free enzyme, could not be recovered completely from plasma after addition to whole blood.[56] These workers suggest that the PEG-enzyme is bound to the blood cells.

This cellular association of PEG-proteins is significant in predicting effects of PEG-protein therapy and in interpreting observed results. Thus it is probable that PEG-proteins associate with cells and do not simply act in plasma.[41,56]

An especially intriguing example of PEG interaction with membranes is the observation by Bittner and colleagues that PEG can be used to fuse the severed halves of invertebrate nerve cells.[57,58] Morphological continuity is demonstrated by transfer of dyes between the fused segments. This work raises the exciting possibility that PEG might be used to fuse severed nerve cells in humans. In a related work, Geron and Meiri have shown that PEG induces fusion of synaptic vesicles and surface membranes of nerve cells.[59]

Clifton and co-workers made the intriguing observation that PEG assists penetration of the blood–brain barrier by vitamin E succinate.[60] This property was used effectively to reduce the damage resulting from mechanical trauma to the brains of rats. If this observation may be generalized to include other animal systems, the use of PEGs could be highly significant for assisting transport of a variety of materials across the blood–brain barrier. Two studies, however, indicate a lack of generality. In the first, Bundgaard and Cserr found that hagfish cerebral capillaries were impermeable to PEG,[61] and in the second, Spigelman *et al.* found that a complex solvent mixture containing PEG 300 had no effect on permeability of the blood–brain barrier.[62] It is important to note, however, that studies of the effects of PEG variables

(molecular weight, end groups, copolymers, covalent linkage, etc.) on this intriguing process have not been conducted.

Finally, we note that the interpretation of many two-phase partitioning results requires the concept that PEG interacts with cell membranes (see Chapters 4–6).

1.3.6. Summary

It is apparent that PEG is of immense utility in addressing a variety of needs in biotechnology and medicine. In addition to the "mainline" applications discussed here there are several others that probably are related to the current undertaking but which, as yet, have not received extensive consideration in the literature. These include use of PEGs in cryoprotection, pharmaceutical preparations, intestinal lavage, tissue culture, and organ protection. In any event, the pertinence of PEG and PEG chemistry to medicine and biology is clear, and it is this author's opinion that the next few years will see a rapid growth in related science and applications. It is hoped that the following chapters will assist in this growth.

ACKNOWLEDGMENTS

The author gratefully acknowledges the many helpful discussions with Allan Hoffman and the financial support of this work by the National Aeronautics and Space Administration and the National Institutes of Health.

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